The Journal of Experimental Biology 213, 2797-2807

doi:10.1242/jeb.040733

Hypercarbic cardiorespiratory reflexes in the facultative air-breathing fish jeju (*Hoplerythrinus unitaeniatus*): the role of branchial CO_2 chemoreceptors

Cheila de Lima Boijink[1], Luiz Henrique Florindo[2,3], Cleo A. Costa Leite[1,3], Ana Lúcia Kalinin[1,3], William K. Milsom[4] and Francisco Tadeu Rantin[1,3,*]

[1]Departament of Physiological Sciences, Federal University of São Carlos, 13565-905 São Carlos, SP, Brazil, [2]Departament of Zoology and Botany, Aquaculture Center (CAUNESP), São Paulo State University – UNESP, 15054-000, São José do Rio Preto, SP, Brazil, [3]National Institute of Science and Technology – Comparative Physiology (FAPESP/CNPq), Brazil and [4]Department of Zoology, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

*Author for correspondence (ftrantin@power.ufscar.br)

Accepted 4 May 2010

SUMMARY

The aim of the present study was to determine the roles that externally *versus* internally oriented CO_2/H^+-sensitive chemoreceptors might play in promoting cardiorespiratory responses to environmental hypercarbia in the air-breathing fish, *Hoplerythrinus unitaeniatus* (jeju). Fish were exposed to graded hypercarbia (1, 2.5, 5, 10 and 20% CO_2) and also to graded levels of environmental acidosis (pH ~7.0, 6.0, 5.8, 5.6, 5.3 and 4.7) equal to the pH levels of the hypercarbic water to distinguish the relative roles of CO_2 *versus* H^+. We also injected boluses of CO_2-equilibrated solutions (5, 10 and 20% CO_2) and acid solutions equilibrated to the same pH as the CO_2 boluses into the caudal vein (internal) and buccal cavity (external) to distinguish between internal and external stimuli. The putative location of the chemoreceptors was determined by bilateral denervation of branches of cranial nerves IX (glossopharyngeal) and X (vagus) to the gills. The data indicate that the chemoreceptors eliciting bradycardia, hypertension and gill ventilatory responses (increased frequency and amplitude) to hypercarbia are exclusively branchial, externally oriented and respond specifically to changes in CO_2 and not H^+. Those involved in producing the cardiovascular responses appeared to be distributed across all gill arches while those involved in the gill ventilatory responses were located primarily on the first gill arch. Higher levels of aquatic CO_2 depressed gill ventilation and stimulated air breathing. The chemoreceptors involved in producing air breathing in response to hypercarbia also appeared to be branchial, distributed across all gill arches and responded specifically to changes in aquatic CO_2. This would suggest that chemoreceptor groups with different orientations (blood *versus* water) are involved in eliciting air-breathing responses to hypercarbia in jeju.

Key words: gills, cardiorespiratory control, hypercarbia, CO_2 and H^+ chemoreceptors, air breathing, jeju, *Hoplerythrinus unitaeniatus*.

INTRODUCTION

Acute exposure of fish to aquatic CO_2 (hypercarbia) typically elicits significant increases in the volume of water pumped with each breath (stroke volume) and/or breathing frequency, resulting in an increase in ventilation volume (Janssen and Randall, 1975; Thomas and Le Ruz, 1982; Smith and Jones, 1982; Burleson and Smatresk, 2000; Reid et al., 2000; Perry and McKendry, 2001; McKendry and Perry, 2001; McKendry et al., 2001; Gilmour, 2001; Milsom et al., 2002; Perry and Reid, 2002). This is usually accompanied by a fall in heart rate (Kent and Peirce, 1978; Perry et al., 1999; Crocker et al., 2000; Reid et al., 2000; Sundin et al., 2000; McKendry and Perry, 2001; McKendry et al., 2001; Perry and Reid, 2002) and an increase in systemic vascular resistance (Perry et al., 1999; McKendry and Perry, 2001). These responses arise from the stimulation of specific CO_2/H^+ chemoreceptors and are not dependent on changes in blood O_2 concentration (Butler and Taylor, 1971; Heisler et al., 1988; Graham et al., 1990; Kinkead and Perry, 1991; Milsom, 1995a; Milsom, 1995b; Perry and Gilmour, 1996; Burleson and Smatresk, 2000; Sundin et al., 2000; Reid et al., 2000; Gilmour, 2001).

At present, the cardiorespiratory responses to CO_2/H^+ in fish are believed to arise primarily, if not exclusively, from receptors distributed throughout the gill arches innervated by the IXth (glossopharyngeal) and Xth (vagus) cranial nerves. It is clear that these receptors in the gills monitor the CO_2 in the water but it is not clear whether they also respond to changes in the CO_2 of arterial blood. There is evidence to suggest both that they do (Janssen and Randall, 1975; Wood and Perry, 1985; Perry and Wood, 1989; Aota et al., 1990; Wood and Munger, 1994; Gilmour and Perry, 1996) and that they do not (Perry et al., 1999; Reid et al., 2000; Sundin et al., 2000; McKendry et al., 2001; Perry and McKendry, 2001; McKendry and Perry, 2001; Perry and Reid, 2002; Gilmour et al., 2005). The data suggest that the receptors responding to external stimuli respond specifically to changes in CO_2 in the water (not pH) (Neville, 1979; Thomas and Le Ruz, 1982; Reid et al., 2000; Sundin et al., 2000) while, to the extent that there is evidence for ventilatory responses arising from receptors responding to internal stimuli, there is a better correlation between the changes in ventilation and changes in arterial pH rather than arterial P_{CO_2} (partial pressure of CO_2) (Heisler et al., 1988; Graham et al., 1990; Wood et al., 1990; Wood and Munger, 1994).

In most species of air-breathing fish, increases in environmental CO_2 also cause initial significant increases in gill ventilation (Johansen and Lenfant, 1968; Perry et al., 2005), and, in some of these species, if the increases in aquatic CO_2 are large enough, they lead to inhibition of gill breathing and stimulate air breathing (Jesse et al., 1967; Johansen et al., 1967; Johansen et al., 1970; Graham

and Baird, 1982; Graham, 1997; Sanchez and Glass, 2001; Sanchez et al., 2005). In other species, however, increases in environmental CO_2 are without effect on gill ventilation (Johansen, 1966; Todd, 1972; McMahon and Burggren, 1987) or air breathing (Johansen et al., 1968; Lomholt and Johansen, 1974). Just as with aquatic CO_2, some air-breathing fish show no response to increasing levels of CO_2 in inspired air (Sanchez and Glass, 2001; Perry et al., 2008) while others show an increase (Smith, 1930; Delaney et al., 1974; Delaney et al., 1976; Delaney et al., 1977; Babiker, 1979) or even a decrease (Jesse et al., 1967) in ventilation of the air-breathing organ (ABO).

The equivocal nature of the data raises questions about the existence and role of internally oriented CO_2/H^+-sensitive chemoreceptors in driving gill ventilation in exclusively water-breathing fish, as well as in driving gill ventilation or air breathing in facultative and obligate air-breathing fish. Clearly much remains to be done to resolve this issue. To date, this has not been examined in any air-breathing species and it will be intriguing to see to what extent similar trends appear in air-breathing holosteans, chondrosteans, teleosts and sarcopterygians. Studies to date regarding the presence of central CO_2/H^+-sensitive chemoreceptors in these groups are inconsistent. Central chemoreceptors have been unequivocally shown to be present in the sarcopterygian lungfish (Sanchez et al., 2001), while there is evidence for their presence in chondrosteans [gar (Wilson et al., 2000)] but not in holosteans [bowfin (Hedrick et al., 1991)].

As a first step in this process, the aim of the present study was to determine the location and the orientation (internal/external) of the CO_2/H^+ chemoreceptors involved in promoting cardiorespiratory responses in a teleost. For this we chose a facultative air breather, the neotropical jeju. We have recently shown that both the gill and air-breathing responses of the jeju to hypoxia involve internally and externally oriented chemoreceptors confined solely to the gills (Lopes et al., 2010) and here we extend these studies to hypercarbic responses. The jeju, *Hoplerythrinus unitaeniatus* [Teleostei, Erytrhinidae (Spix and Agassiz, 1829)] is an active freshwater predator found in streams and shallow waters throughout South America. During periods of drought this species remains confined to marginal lagoons with stagnant hypoxic, hypercarbic waters for weeks to months. The ABO of jeju is the swim bladder, which is subdivided into an anterior and a posterior section. The posterior section is further subdivided into an anterior, richly vascularized respiratory portion and a non-respiratory posterior sac (for details, see Glass and Rantin, 2009). As a facultative air breather, the jeju relies primarily on its gills for gas exchange, as long as the water is normoxic/normocarbic, but uses its ABO as a facultative option during severe environmental hypoxia/hypercarbia (Carter and Beadle, 1931; Farrell, 1978; Kramer et al., 1978; Smith and Gannon, 1978; Stevens and Holeton, 1978; Graham, 1997; Oliveira et al., 2004).

MATERIALS AND METHODS

Experimental animals

Adult jeju, *H. unitaeniatus*, weighing 253±15 g (mean ± s.e.m.) were collected in the Paraná River Basin, near Bataguaçu, Mato Grosso do Sul, Brazil. In the laboratory, fish were maintained in 10001 holding tanks supplied with a continuous flow of dechlorinated and aerated water [normoxic conditions, $P_{O_2} \geq 130$ mmHg (17.29 kPa)] at a constant temperature (25°C). Fish were fed with live food (smaller fish of various species) *ad libitum*. Food, however, was withheld for 2–3 days before trials. All experiments were conducted according to the rules of the Brazilian College on Ethics in Animal Experimentation – COBEA, meeting all the regulations and ethical guidelines in Brazil.

Animal preparation

For surgical procedures fish were anesthetized by immersion in a solution of benzocaine (0.1 g l^{-1}) and transferred to a surgical table where their gills were continuously irrigated with a weaker solution of benzocaine (0.05 g l^{-1}) that was constantly aerated to maintain adequate levels of O_2. Two polyethylene cannulae (PE 100 and PE 60) were introduced into the buccal cavity through a hole in the dorsal palate to record buccal pressure (*P*buccal) [to determine ventilatory frequency (f_R) and amplitude (V_{AMP})] and for bolus injections of water equilibrated with different levels of CO_2 or containing different [H^+]. The caudal artery and vein were also cannulated (PE 50) (Axelsson and Fritsche, 1994) to record arterial pressure (*P*a) and heart rate (f_H), and for intravenous injections of CO_2 or H^+-equilibrated saline (0.9% NaCl, 100 i.u. ml^{-1} heparin), respectively.

The operculum was reflected forward under a stereoscopic microscope (Opto SM 2001, Opto Electronics, São Carlos, SP, Brazil) and a small incision (2 cm) was made in the epithelium above the first and second gill arches where they meet the roof of the opercular cavity. This allowed us to access and denervate the appropriate nerve branches to make up four experimental groups: an Intact group (I), consisting of non-operated animals (N=7) and sham-operated animals (N=3) that had their nerves exposed but not sectioned; an IX group (N=10), consisting of animals having the branchial branch of the IXth cranial nerve sectioned; a G1 group (N=10), consisting of animals having the branchial branch of the IXth and the first branch of the Xth cranial nerve sectioned completely denervating the first gill arch; and a G4 group (N=10), consisting of animals having the branchial branch of the IXth and the branchial branches of the Xth nerves to all four gill arches sectioned. The visceral and cardiac branches of the vagus were kept intact. The healing process in jeju was rapid, and the incisions were covered with 'scar tissue' within about 24 h. This species does not have a pseudobranch and all denervations were confirmed 'post mortem'.

Experimental protocol

Series 1: gill ventilation and associated cardiovascular changes

After recovering from the surgery, animals were placed in individual plastic tubes covered at both ends with mesh and placed in a large chamber with a constant flow of aerated water at 25°C where they remained undisturbed for a minimum of 24 h before beginning the experimental protocol. In this series of experiments the fish did not have access to air to eliminate the confounding effects of air breathing on interpretation of gill ventilatory responses. Their cannulae were connected to pressure transducers to record *P*buccal and *P*a, and to evaluate f_R, V_{AMP}, *P*a and f_H, respectively. The pressure transducers were calibrated manometrically, connected to pre-amplifiers and the outputs monitored and recorded with a data acquisition system (Dataq DI-194, DATAQ Instruments, Inc., Akron, OH, USA) recording at 125 Hz per channel. The partial pressure of O_2 in water ($P_{W_{O_2}}$) was constantly monitored with an O_2 electrode (FAC 001) connected to an O_2 analyzer (FAC 204, FAC Instruments, São Carlos, SP, Brazil), calibrated with a borax solution (0.01 N) saturated with sodium sulphite and aerated water ($P_{W_{O_2}}$=0 and 140 mmHg, respectively). The water pH was constantly monitored.

Injections of CO_2-equilibrated solutions

Each intact or denervated fish (N=40) was submitted to a series of internal (saline *via* the cannula implanted in the caudal vein) or

external (water *via* the buccal cannula) injections of CO_2-equilibrated solutions. Internal injections preceded external injections and were performed in the following order: (1) 0.3 ml saline; (2) 0.3 ml saline equilibrated with 5% CO_2; (3) 0.3 ml saline equilibrated with 10% CO_2; (4) 0.3 ml saline equilibrated with 20% CO_2; (5) 1.5 ml water; (6) 1.5 ml water equilibrated with 5% CO_2; (7) 1.5 ml water equilibrated with 10% CO_2; and (8) 1.5 ml water equilibrated with 20% CO_2. Solutions (saline/water) were equilibrated to each CO_2 level by bubbling with gases of specific CO_2 percentages for about 15 min. After each injection the cardiorespiratory variables were recorded for 3 min or until the f_R, f_H and Pa returned to the pre-injection values.

The pH values of each saturated solution were measured and the values (means ± s.e.m.) obtained were: 5.12±0.07 (saline + 5% CO_2), 4.90±0.02 (saline + 10% CO_2), 3.72±0.03 (saline + 20% CO_2), 5.63±0.02 (water equilibrated with 5% CO_2), 5.33±0.06 (water equilibrated with 10% CO_2), and 4.70±0.03 (water equilibrated with 20% CO_2).

Injections of acid solutions

To differentiate between the cardiorespiratory responses arising from the direct effects of CO_2 and the indirect effects of decreasing pH caused by the increases in CO_2 concentrations, both saline and water were titrated with HCl (3, 4 and 5 nmol l^{-1} HCl, respectively) to reach the same pH values as those recorded for the hypercarbic solutions (for internal saline injections these were pH=5.1, 4.9 and 3.7; for external water injections these were pH=5.6, 5.3 and 4.7). These solutions were injected in the same sequence as for the CO_2 injections into the same group of animals (N=40) and the cardiorespiratory variables were recorded following the same procedure.

Exposure to graded changes in environmental CO_2

After the internal and external injections, the same animals (N=40) were exposed to different levels of environmental hypercarbia. These different hypercarbic levels were obtained by bubbling the water of a gas dispersion column with known CO_2 mixtures. The water was fully saturated after bubbling with each CO_2 concentration for 15 min. The water equilibrated with CO_2 was subsequently directed to the experimental tank. Fish were exposed to the CO_2 concentrations of 1, 2.5, 5, 10 and 20% for 15 min each. The cardiorespiratory variables (f_R, V_{AMP}, f_H and Pa) were recorded during the last 10 min at each hypercarbic level (1, 2.5, 5, 10 and 20% CO_2). The pH of the water was also monitored with a pH electrode (Mettler-Toledo A1844, Mettler-Toledo AG, São Paulo, Brazil) connected to a pH meter (Quimis 400A; Quimis Scientific Apparatus, Diadema, SP, Brazil) and the values were: pH 7.0–6.8 (normocarbia), 6.0 (1% CO_2), 5.8 (2.5% CO_2), 5.6 (5% CO_2), 5.3 (10% CO_2), and 4.7 (20% CO_2). The partial pressure of O_2 (P_{O_2}) levels during the hypercarbic exposures were: 137±0.05 (1%), 135±0.07 (2.5%), 130±0.06 (5%), 123±0.05 (10%), and 118±0.07 (20%) mmHg.

Exposure to graded changes in environmental acidification

A further group of animals (N=10) was exposed to graded decreases in environmental pH. The different pH levels were obtained by titrating the water with HCl to reach levels equivalent to those measured during exposure to the different levels of hypercarbia; pH 7.0–6.8 (normocarbia), 6.0 (1% CO_2), 5.8 (2.5% CO_2), 5.6 (5% CO_2), 5.3 (10% CO_2), and 4.7 (20% CO_2). They were exposed to each pH level for 15 min and the cardiorespiratory variables (f_R, V_{AMP}, f_H and Pa) were recorded during the last 10 min at each level.

Series 2: air breathing

This series of experiments utilized an experimental setup similar to that described by Rantin and colleagues (Rantin and Kalinin, 1996; Rantin et al., 1998) to allow fish access to air. The system consisted of two chambers: an upper compartment, where the fish was kept during the experiment, and a lower compartment, serving to gas the water with CO_2. The water was continuously re-circulated from the lower to the upper compartment. The shape of the upper chamber allowed fish to remain on the bottom or move up to the surface to air breathe while restricting lateral movements. All cannulae were connected and instruments were calibrated as described above. Air breaths were measured from both the video recordings of the behavior of the fish as well as from the buccal pressure trace.

Exposure to graded changes in environmental CO_2

Individuals of all four groups of fish (I, IX, G1 and G4; N=10 each) were subjected to progressive hypercarbia, as described above, and their air-breathing frequency and time spent in aerial respiration were quantified at each of the graded changes in CO_2 tension.

Exposure to graded changes in environmental pH

The group of animals (N=10) exposed to graded decreases in environmental pH described above were also subjected to the same protocol in this experimental apparatus to determine whether there were any effects of environmental acidification, without changes in CO_2 concentration, on air breathing.

Data analysis

All procedures were carried out in sequence on the same day to maintain patent catheters and minimize time of cannulation, starvation, confinement, etc. The cardiovascular and respiratory variables were measured 10 s before each injection and at 10, 20, 30, 40, 50, 60, 120, 180 and 240 s after each injection. The measurements of f_H and f_R are expressed as beats min^{-1} and breaths min^{-1}, respectively. The total ventilation (V_{TOT}) was determined as $V_{TOT}=V_{AMP}\times f_R$. V_{AMP}, Pa and V_{TOT} are expressed as the percentage change from basal resting levels. Air-breathing frequency and time spent in air breathing (i.e. at the surface engaged in air-breathing activity) are expressed in absolute values averaged over the last 10 min of each exposure.

Data are presented as means ± standard error of the mean (±s.e.m.). Differences between initial levels of each variable and those following the injections or during the hypercarbic or acid exposures both within and between groups (intact and denervated) were determined using a two-way repeated-measures analysis of variance (ANOVA) followed by a Dunnett's multiple comparison test. The commercial package SigmaStat v3.0 (SPSS Inc., Chicago, IL, USA) was used to carry out statistical analyses, and the fiducial limit of significance in all cases was 5% (P<0.05).

RESULTS

Series 1: gill ventilation and associated cardiovascular changes

The effects of selective denervation on resting cardiorespiratory variables

Progressive bilateral section of cranial nerve IX and branches of nerve X to the gills significantly elevated resting values of f_H. There was a tendency for Pa to decrease with progressive denervation but this was not significant. There were no significant changes in any of the resting values for the ventilatory variables (Table 1).

Table 1. Resting values of the cardiorespiratory variables of intact and denervated (IX, G1 and G4) jeju, *Hoplerythrinus unitaeniatus*

Variable	Intact	IX	G1	G4
f_H (beats min^{-1})	54.6±3.4	72.0±6.1*	70.2±3.6*	72.0±4.3*
*P*a (%)	0.0±0.0	–2.3±10.2	–7.1±12.1	–14.4±9.0
f_R (breaths min^{-1})	73.2±3.4	69.0±3.3	68.4±4.2	64.2±2.0
V_{AMP} (%)	0.0±0.0	29.3±8.3	38.7±14.4	32.3±16.6
V_{TOT} (%)	0.0±0.0	22.4±11.6	30.5±13.0	12.9±12.2

f_H, heart rate; *P*a, arterial blood pressure; f_R, respiratory frequency; V_{AMP}, ventilation amplitude; V_{TOT}, total ventilation.
Values are means ± s.e.m.
*Significant difference in relation to the intact group (P<0.05).

Injections of CO_2-equilibrated solutions

Internal injections into the caudal vein of saline equilibrated with 5, 10 and 20% CO_2 produced small, transient increases in *P*a, f_R and V_{AMP} and decreases in f_H that were noticeably larger with increasing levels of CO_2; however, these measurements were never significant in either the intact or the denervated fish (data not shown).

External injections of water into the buccal cavity equilibrated with 5, 10 and 20% CO_2, however, produced a significant decrease in f_H that became more pronounced and continuous with increasing levels of CO_2 (Fig. 1A,C,E). The responses were fast, peaking around 20–40 s after the injections and returning gradually to basal levels by about 2 min. This response was abolished by denervation of the IXth nerve alone at low levels of inspired CO_2 (Fig. 1A). It was reduced following denervation of the IXth nerve (IX group) and the first gill arch (G1) and abolished by total gill denervation (G4 group) at higher levels of inspired CO_2 (Fig. 1B,C).

The effects of buccal injections of water equilibrated with CO_2 on *P*a are depicted in Fig. 1B,D,F. There was a progressive increase in blood pressure with injections of water equilibrated with increasing levels of CO_2 in the intact and IX groups. In the G1 group, a significant increase in *P*a only occurred in response to 20% CO_2 and all injections failed to produce significant changes in the G4 group.

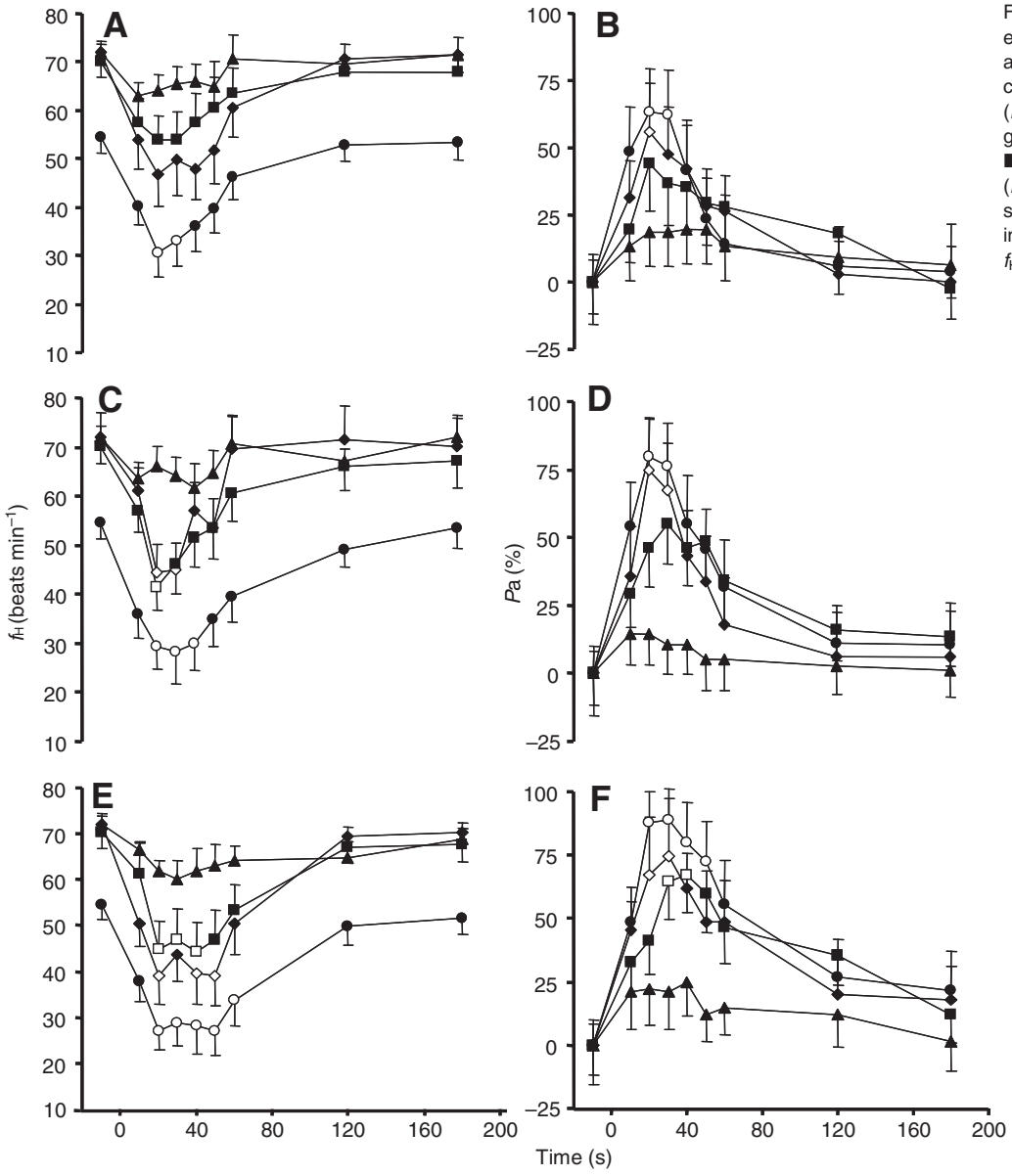

Fig. 1. Effects of external injections equilibrated with 5% (A and B), 10% (C and D) and 20% (E and F) CO_2 on the cardiac responses (f_H and *P*a) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (N=10); ◆, Group IX (N=10); ■, Group G1 (N=10); ▲, Group G4 (N=10). Open symbols represent significant differences in relation to the initial values. Points are means ± s.e.m. f_H, heart rate; *P*a, arterial pressure.

Fig. 2 illustrates the effects of external injections of water equilibrated with 5, 10 and 20% CO_2 on f_R (Fig. 2A,C,E) and V_{AMP} (Fig. 2B,D,F). While all levels of CO_2 injected produced significant increases in both variables in the intact group, only the injections equilibrated with 10 and 20% CO_2 produced significant effects in the IX group and, despite a tendency to increase, no significant effects were detected in the G1 and G4 groups. As a consequence of these responses, there was a significant increase in V_{TOT} at all levels of injected CO_2 in the I and IX groups. There was also a significant effect of the 10 and 20% CO_2 injections in the G1 group but complete gill denervation eliminated the response to all CO_2 injections (Fig. 3).

Injections of acid solutions

Internal and external injections of saline and water titrated with HCl to the same pH levels as the CO_2 injections failed to produce any significant changes in any of the cardiorespiratory variables (Table 2).

Exposure to graded changes in environmental CO_2

The effects of hypercarbia (1, 2.5, 5, 10 and 20% CO_2) on f_H and Pa of intact and denervated fish are presented in Fig. 4. In the intact fish, exposure to 1 and 2.5% CO_2 did not cause any significant change in f_H or Pa; however, when the intact group was exposed to 5, 10 and 20% CO_2, f_H decreased and Pa increased significantly. This pronounced bradycardia and hypertension was also observed in the IX and G1 groups when exposed to the higher levels of CO_2. Total gill denervation (G4) completely abolished the bradycardia and hypertension in fish exposed to all levels of CO_2.

Fig. 5 reveals that progressive hypercarbia produced a bimodal response in all respiratory variables in intact and partially denervated fish but not in completely denervated animals. Thus, in the I and IX groups, f_R and V_{AMP} increased during exposure to 5% CO_2, and there was a trend for these variables to increase in the G1 group such that V_{TOT} increased in all of the I, IX and G1 groups. Subsequent exposure to 10 and 20% CO_2, however, caused a significant decrease in the f_R of the I, IX and G1 groups, to below

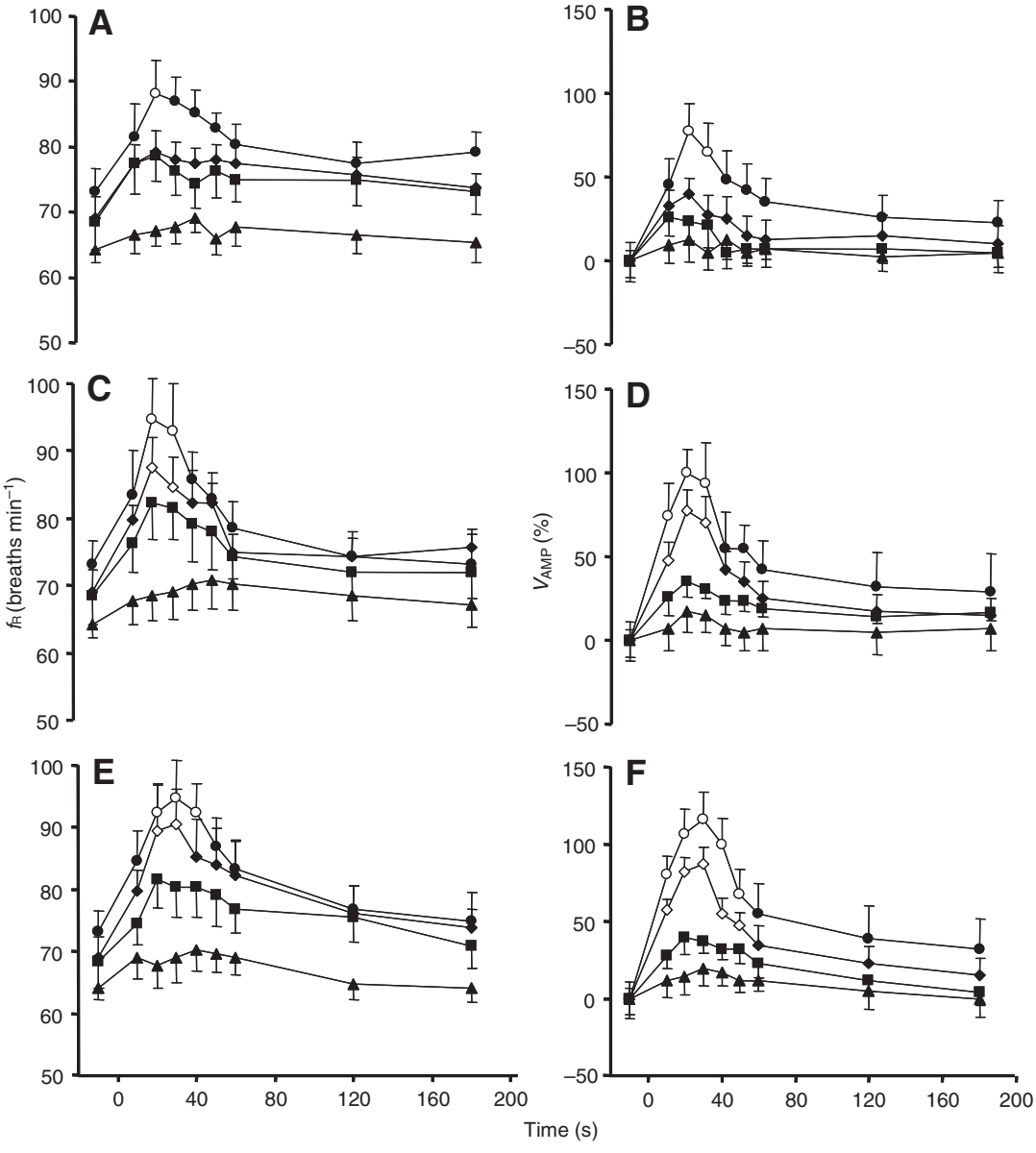

Fig. 2. Effects of external injections equilibrated with 5% (A and B), 10% (C and D) and 20% (E and F) CO_2 on the respiratory responses (f_R and V_{AMP}) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (N=10); ◆, Group IX (N=10); ■, Group G1 (N=10); ▲, Group G4 (N=10). Open symbols represent significant differences in relation to the initial values. Points are means ± s.e.m. f_H, heart rate; V_{AMP}, ventilation amplitude.

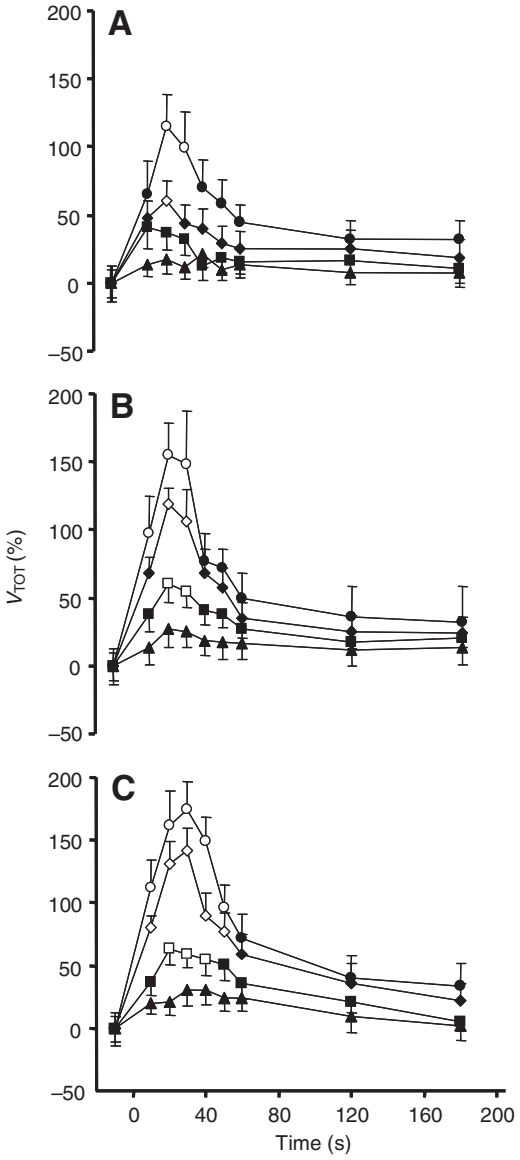

Fig. 3. Effects of external injections equilibrated with 5% (A), 10% (B) and 20% (C) CO_2 on the respiratory responses (V_{TOT}) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (N=10); ◆, Group IX (N=10); ■, Group G1 (N=10); ▲, Group G4 (N=10). Open symbols represent significant differences in relation to the initial values (normocarbia). Points are means ± s.e.m. V_{TOT}, total ventilation.

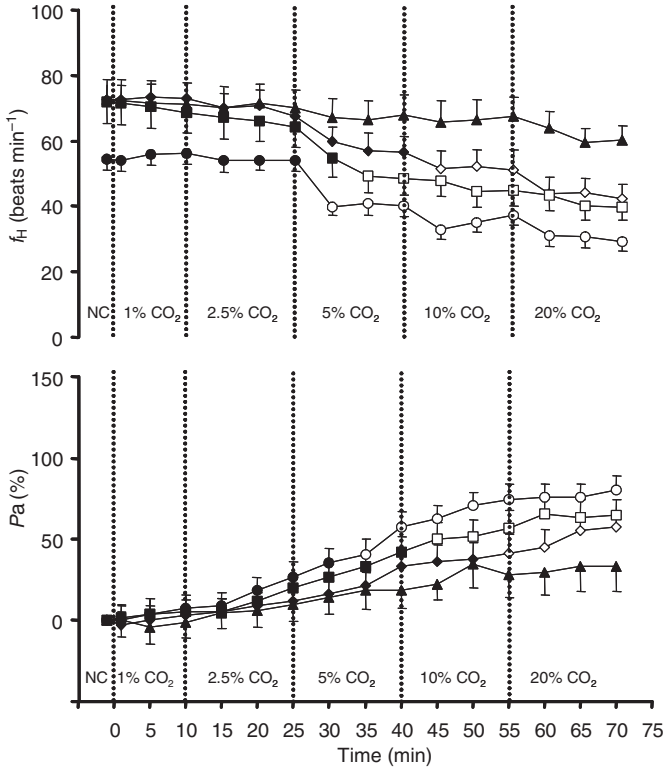

Fig. 4. Effects of graded hypercarbia (1, 2.5, 5, 10 and 20% CO_2) on the heart frequency (f_H) and arterial blood pressure (Pa) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (N=10); ◆, Group IX (N=10); ■, Group G1 (N=10); ▲, Group G4 (N=10). Open symbols represent significant differences in relation to the initial values (NC=normocarbia). Points are means ± s.e.m.

starting levels. It also caused V_{AMP} and V_{TOT} to return to normocarbic levels (or below normocarbic levels in the case of V_{TOT} for the G1 group). Of note, f_R, V_{AMP} and V_{TOT} of the completely denervated fish were maintained at normocarbic values throughout, indicating that both the initial increases in f_R, V_{AMP} and V_{TOT} at 5% CO_2 as well as the subsequent decreases at the higher levels of CO_2 required intact branchial innervation.

Exposure to graded changes in environmental pH

Just as with the internal and external injections of saline and water titrated with HCl, exposure of fish to progressive decreases in aquatic pH to the same pH levels produced by the graded increases in CO_2 failed to produce any significant changes in any of the cardiorespiratory variables (data not shown).

Series 2: air breathing

Exposure to graded changes in environmental CO_2

The effects of graded hypercarbia on aerial respiration frequency (f_{RA}) and time spent in aerial respiration (T_{RA}) are presented in Fig. 6. Fish did not perform aerial respiration in normocarbia nor when exposed to 1, 2.5 or 5% CO_2. Aerial respiration, however, was recorded for all groups exposed to 10% CO_2, although exposure to 20% CO_2 did not increase air breathing further. The number of breaths taken every 10 minutes was progressively reduced by increasing gill denervation. While the one breathe per 10 minute interval taken by the G4 group on 10% CO_2 was significant, it was not when these fish were exposed to 20% CO_2.

Exposure to graded changes in environmental pH

Just as the exposure of fish to progressive decreases in aquatic pH (to the same pH levels produced by the graded increases in CO_2) failed to produce any significant changes in any of the cardiorespiratory variables, it also failed to produce any aerial respiration.

DISCUSSION

Series 1: gill ventilation and associated cardiovascular changes

Effects of the selective denervation on the cardiorespiratory variables

In both this and a previous study on jeju (Lopes et al., 2010), progressive gill denervation led to an increase in resting values of

Table 2. Effects of external H^+ injections on the cardiorespiratory variables of intact and denervated (IX, G1 and G4) jeju, *Hoplerythrinus unitaeniatus*

Variable	Pre	Post 3 mmol l^{-1}	Post 4 mmol l^{-1}	Post 5 mmol l^{-1}
f_H (beats min^{-1})	52.6±3.2	51.0±3.1	49.8±2.8	50.4±3.2
*P*a (%)	0.0±0.0	5.7±6.6	3.3±5.9	6.7±7.1
f_R (breaths min^{-1})	69.6±4.2	78.0±5.0	76.2±4.2	76.8±5.4
V_{AMP} (%)	0.0±0.0	9.4±3.7	8.1±4.8	9.6±6.2
V_{TOT} (%)	0.0±0.0	7.8±6.6	8.5±5.9	9.7±6.3

f_H, heart rate; *P*a, arterial blood pressure; f_R, respiratory frequency; V_{AMP}, ventilation amplitude; V_{TOT}, total ventilation.
Pre, immediately before the injections; post, 20 s after the injections.
Values are means ± s.e.m.

f_H. Because progressive denervation removes both afferent and efferent innervation of the gills, it is difficult to determine if this post-denervation tachycardia is related to a decrease in the control of branchial vascular resistance, removal of sensory afferent information or both. However, the progressive denervation had no significant effect on any of the respiratory variables, suggesting that there is no significant sensory input arising from the gills under normoxic, normocarbic conditions modulating respiratory drive. Similar observations have been made by others on other species (Sundin et al., 1999; Reid et al., 2000).

The CO_2 threshold for eliciting cardiorespiratory responses

The increase in aquatic CO_2 required to elicit a ventilatory response (the threshold level) varies greatly between species. Jeju, along with such species as eel, carp, tambaqui and traíra require fairly high levels of CO_2 (≥5% CO_2) before they respond (Reid et al., 2000; Soncini and Glass, 2000; Sundin et al., 2000), while others (zebrafish, trout) are very sensitive, responding to very small changes in aquatic CO_2 (<1% CO_2) (Thomas, 1983; Perry et al., 1999; Gilmour, 2001; Vulesevic et al., 2006). It has been suggested that this reflects the tolerance of each species to CO_2, with ventilatory responses to CO_2 only being elicited at environmentally appropriate levels (Gilmour, 2001). Jeju experience hypercarbic waters when they are confined to marginal lagoons and ponds during yearly drought periods where pH may fall as low as 3.5 (Sioli, 1984).

Do the chemoreceptors respond to changes in CO_2 or $[H^+]$?

Based on our data comparing (internal and external) bolus injections of CO_2-equilibrated solutions with bolus injections of normocarbic solutions with identical pH, as well as comparing the results of the progressive graded changes in CO_2 with graded changes in acid solutions of identical pH, we can say unequivocally that jeju respond to changes in CO_2 *per se* and not to changes in extracellular $[H^+]$. Similar results have been shown for dogfish (McKendry et al., 2001), salmonids (Janssen and Randall, 1975; Neville, 1979; Thomas and Le Ruz, 1982; McKendry et al., 2001), traíra (Reid et al., 2000) and tambaqui (Sundin et al., 2000). Although these receptors may only respond to changes in aquatic CO_2 and not [H+], however, at the cellular level they may still do this by responding to CO_2-induced changes in intracellular pH as has also been suggested in mammals (Gonzalez et al., 1994; Nattie, 1999).

Do the chemoreceptors driving gill ventilation respond to changes in CO_2 in the water or the blood?

Determining whether the responses of fish to increasing levels of aquatic CO_2 are due to stimulation of receptors that sense water

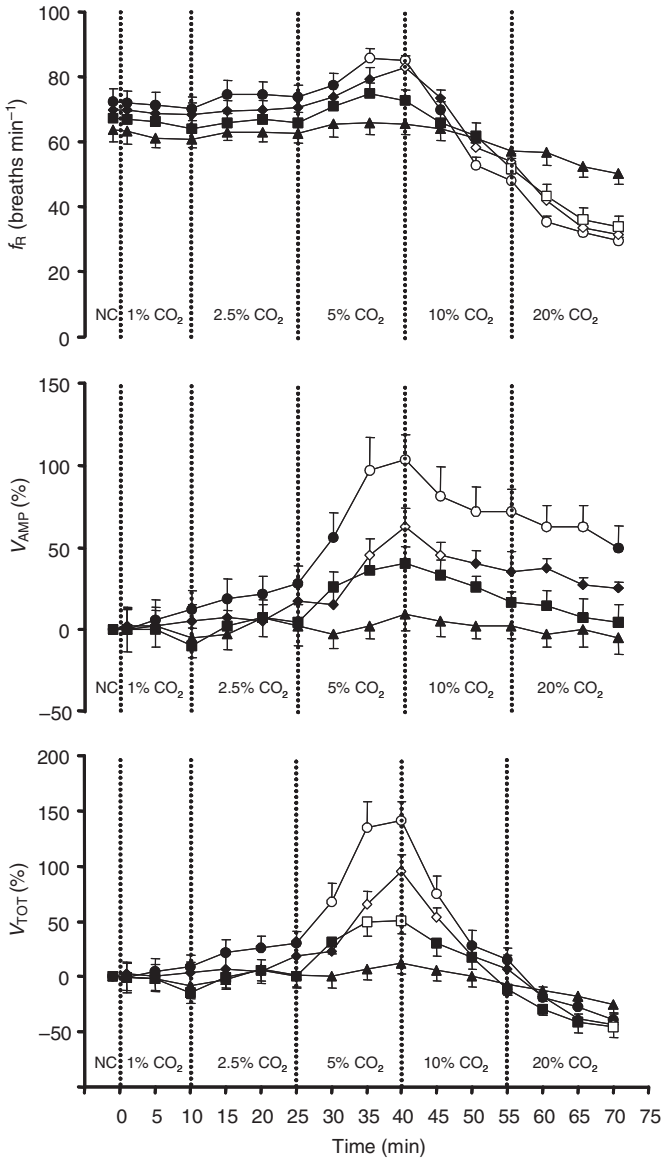

Fig. 5. Effects of graded hypercarbia (1, 2.5, 5, 10 and 20% CO_2) on the respiratory responses (f_R, V_{AMP} and V_{TOT}) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (*N*=10); ◆, Group IX (*N*=10); ■, Group G1 (*N*=10); ▲, Group G4 (*N*=10). Open symbols represent significant differences in relation to the initial values (NC=normocarbia). Points are means ± s.e.m. f_R, ventilation frequency; V_{AMP}, ventilation amplitude; V_{TOT}, total ventilation.

versus blood has not been straightforward. Just as aquatic hypercarbia will lead to changes in the P_{CO_2} (hypercapnia) of arterial blood in fish, external injections of CO_2-equilibrated water into the buccal cavity may also diffuse into the gills and stimulate internal, blood-sensing chemoreceptors. By the same token, intravenous or intra-arterial injections of CO_2-equilibrated saline may diffuse out through the gills and stimulate externally oriented, water-sensing chemoreceptors. For the most part, however, intra-arterial injections of CO_2/H^+ fail to modulate any of the cardiorespiratory variables. While intra-arterial injections of CO_2/H^+ stimulated ventilation in rainbow trout in some studies (Janssen and Randall, 1975; Aota et al., 1990; Gilmour and Perry, 1996), in other studies they had no effect on ventilation in trout, or in dogfish, salmon, tambaqui and

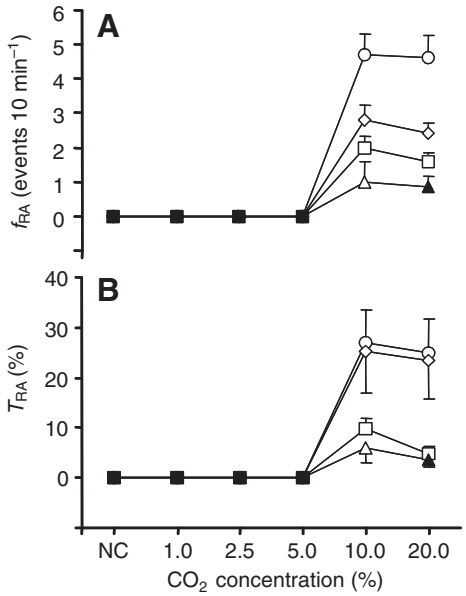

Fig. 6. Effects of graded hypercarbia (1, 2.5, 5, 10 and 20% CO_2) on the aerial respiration frequency (f_{RA}) (A) and time spent in aerial respiration (T_{RA}) (B) of jeju (*Hoplerythrinus unitaeniatus*). ●, Intact group (N=10); ◆, Group IX (N=10); ■, Group G1 (N=10); ▲, Group G4 (N=10). Open symbols represent significant differences in relation to the initial values (NC=normocarbia). Points are means ± s.e.m.

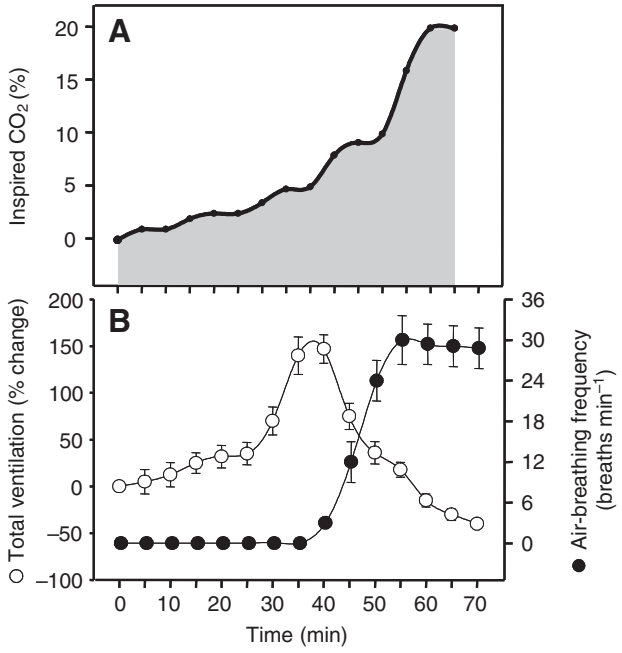

Fig. 7. Effects of graded hypercarbia (1, 2.5, 5, 10 and 20% CO_2) (A) on gill ventilation (V_{TOT}) (B, left-hand axis) and aerial respiration frequency (f_{RA}) (B, right-hand axis) of jeju (*Hoplerythrinus unitaeniatus*). Points are means ± s.e.m. Data were collected from separate groups of fish but the data are combined here and expressed as a function of time during the exposure protocol to demonstrate the correlation between the levels of hypercarbia at which aerial respiration begins to increase and gill ventilation begins to decline.

traíra (Gilmour and Perry, 1996; Reid et al., 2000; Sundin et al., 2000; McKendry et al., 2001). They also had no effect in jeju in the present study. These data strongly implicate water-sensing chemoreceptors as the primary, if not exclusive, source of sensory information giving rise to these responses. In this context, injections of the carbonic anhydrase inhibitor, acetazolamide, that lead to CO_2 retention and blood acidification also do not alter ventilation in dogfish, trout and tambaqui (Perry and McKendry, 2001; Perry and Reid, 2002; Gilmour et al., 2005). A further implication of these results is that jeju lack central CO_2/H^+ chemoreceptors involved in eliciting cardiovascular or gill ventilatory reflexes. This is totally speculative as it is also possible that the internal injections of CO_2/acid stimuli were 'diluted' or buffered as the blood flowed from the injection site in the tail to the brain. While this is undoubtedly true for the CO_2 injections (the bolus of CO_2 could easily have been excreted across the gills), it is less likely for the acid injections and, to the extent that there is evidence for ventilatory responses arising from receptors responding to internal stimuli, there is a better correlation between the changes in ventilation and changes in arterial pH rather than arterial P_{CO_2} (Heisler et al., 1988; Graham et al., 1990; Wood et al., 1990; Wood and Munger, 1994). This conclusion remains to be directly tested.

What are the locations of the CO_2-sensing chemoreceptors involved in the different cardiorespiratory responses?

In jeju, progressive denervation of the cranial nerves (IX and X) supplying the gill arches indicates that CO_2/H^+ chemoreceptors are located exclusively in the gills because total gill denervation abolished all of the cardiorespiratory responses we evaluated. Similar results have been obtained in several other studies (reviewed by Milsom, 1995a; Milsom, 1995b; Milsom, 2002; Gilmour, 2001; Perry and Gilmour, 2002; Gilmour and Perry, 2007).

Hypercarbia has now been shown to elicit bradycardia in several but not all species. It has been reported in dogfish (Kent and Peirce, 1978; McKendry et al., 2001), rainbow trout (Perry et al., 1999; Mckendry and Perry, 2001; Perry and Reid, 2002), traíra (Reid et al., 2000) tambaqui (Sundin et al., 2000; Florindo et al., 2004) and now jeju (present study). However, species like catfish (Burleson and Smatresk, 2000), white sturgeon (Crocker et al., 2000) and American eel (Perry and Gilmour, 2002) do not display bradycardia in response to hypercarbia. Our data strongly suggest that the cardiac responses in jeju are mediated by receptors located on all gill arches, although all we can say for certain is that they are located on more than the first gill arch. This was also the case in traíra (Reid et al., 2000), although in trout and tambaqui the hypercarbic bradycardia was mediated exclusively by receptors located in the first gill arch (Sundin et al., 2000; McKendry and Perry, 2001; Perry and Reid, 2002).

Exposure to hypercarbia has also been shown to elicit hypertension in tambaqui and trout as it did here in jeju (Perry et al., 1999; Sundin et al., 2000; McKendry and Perry, 2001; Florindo et al., 2004) but to elicit hypotension in traíra (Reid et al., 2000). Interestingly, the hypotension in traíra was eliminated by denervation of the IXth nerve alone (Reid et al., 2000) while elimination of the hypertension required denervation of all gill arches in jeju, as well as extra-branchial receptors in tambaqui (Sundin et al., 2000; Florindo et al., 2004). The hypertension has been attributed to a reflex increase in systemic vascular resistance in trout and tambaqui (Perry et al., 1999; Sundin et al., 2000; McKendry and Perry, 2001) and to a reflex vasodilation in traíra (Reid et al., 2000). The basis and significance of the large inter-specific variability shown in this response remains unclear (Perry and Gilmour, 2002).

Jeju significantly increased f_R, V_{AMP} and V_{TOT} in response to 5% CO_2 in agreement with other studies in a variety of fish species such as dogfish, *Scyliorhinus stellaris* (Heisler et al., 1988; McKendry et al., 2001), skate, *Raja ocellata* (Graham et al., 1990; Wood et al., 1990), rainbow trout (Kinkead and Perry, 1991; Gilmour and Perry, 1994; Perry et al., 1999), catfish (Burleson and Smatresk, 2000), traíra (Reid et al., 2000) and tambaqui (Sundin et al., 2000; Milsom et al., 2002).

Our data indicate that the ventilatory responses are mediated by chemoreceptors located primarily, if not exclusively, on the first gill arch. Those eliciting reflex increases in f_R appear to be located on all gill arches in traíra (Reid et al., 2000) and tambaqui (Sundin et al., 2000) while those eliciting reflex increases in V_{AMP} in traíra may be located elsewhere in the orobuccal cavity (Reid et al., 2000; Sundin et al., 2000) as has also been suggested for extra-branchial O_2 chemoreceptors (Hughes and Shelton, 1962; Butler et al., 1977; Sundin et al., 1999).

Series 2: depression of gill ventilation at high levels of CO_2 and stimulation of air breathing

While exposure to 5% CO_2 stimulated ventilation in jeju, exposure to higher levels of CO_2 in the water led to a significant reduction in gill ventilation. It also stimulated air breathing (Fig. 7). Similar responses occur in most air-breathing fish. While there have been some studies in which increases in environmental CO_2 were without effect on gill ventilation (Johansen, 1966; Todd, 1972; McMahon and Burggren, 1987) or air breathing (Johansen et al., 1968; Lomholt and Johansen, 1974), in most species, significant increases in gill ventilation occur in response to low levels of aquatic CO_2 (<3%) (Johansen and Lenfant, 1968; Perry et al., 2005) while further increases in aquatic CO_2 (>2–10%) lead to inhibition of gill breathing and stimulate air breathing (Johansen et al., 1967; Johansen et al., 1970; Graham and Baird, 1982; Jesse et al., 1967; Graham, 1997; Sanchez and Glass, 2001; Sanchez et al., 2005). While the high levels of CO_2 used in the present study might raise concerns about the possibility of the CO_2 producing anesthetic effects in these fish (Bernier and Randall, 1998), the tight correlation between the decline in gill ventilation and the onset of air breathing suggests otherwise. Furthermore, the full expression of both required intact gills and the more intact the fish, the greater the initial response to low levels of CO_2 and the greater the depression with high levels of CO_2. This suggests that the decline in gill ventilation was not a direct or depressive effect of the high CO_2 but was reflexively mediated. The lack of any further increase in air breathing on 20% CO_2 (indeed there was a small non-significant decline) and the further falls in the gill respiratory variables might reflect an interaction between central depressive effects and peripheral stimulation but the overall conclusions remain the same.

Identifying the location of the receptors involved in producing this coordinated response is somewhat problematic. While complete gill denervation eliminated the depression in gill ventilation and air breathing in fish exposed to 20% CO_2, there was still a very small air-breathing response (one breath every 10 min or 20% of the response seen in intact fish) that was significant in the completely denervated fish breathing 10% CO_2. These low levels of air breathing occurred in 5 out of 10 fish whose gill denervation was confirmed separately by two individuals. Three of the fish took only one breath every 10 min while the remaining two fish took two and three. This indicates that a small percentage of the air breathing in response to hypercarbia is either mediated by extra-branchial chemoreceptors at some unknown location or by some non-specific stimulus. It should be noted that the bolus injections that were administered to determine the relative roles of internal *versus* external chemoreceptors were only administered to fish without access to air. Complete gill denervation eliminated all of these responses. This raises the possibility that the extra-branchial sites involved in producing the remaining air-breathing response could be internal. Wherever these receptors are located (and whatever they are responding to), however, their contribution is relatively small.

Just as with gill ventilation, comparing the results of the progressive graded changes in CO_2 with graded changes in acid solutions of identical pH on air breathing, we can say unequivocally that jeju respond to changes in CO_2 *per se* and not to changes in extra-cellular $[H^+]$.

These data raise several intriguing possibilities, all of which remain to be tested. The first is that if aquatic hypercarbia is acting primarily, if not exclusively, *via* water-sensing CO_2 receptors in the gills, then either of two things must occur. The first is that low levels of afferent input stimulate gill ventilation initially but increasing levels of input are transformed centrally (in the brain) to excite air breathing and inhibit water breathing. The other is that there are populations of chemosensitive cells with different stimulus thresholds that project to different areas of the brain such that the input from the high threshold receptors overrides the input from the low threshold receptors to produce the switch to air breathing.

The second intriguing suggestion to arise from the data is that the receptors that give rise to air breathing in response to hypercarbia are different from those that give rise to air breathing in response to hypoxia. It has now been shown in jeju that internally oriented receptors sensing changes in O_2 levels in the blood play the predominant role in eliciting air breathing in response to hypoxia and that these receptors are exclusively located in the gills (Lopes et al., 2010). Up until now, the majority of the data have indirectly indicated that the same receptor cells might be involved in sensing both hypoxia and hypercarbia, much as they do in the carotid bodies of mammals (e.g. Smatresk, 1990; Hempleman et al., 1992; West and Van Vliet, 1993; Gonzalez et al., 1994; Peers and Buckler, 1995; Kusakabe, 2002). The current data would suggest that this is not necessarily the case. These intriguing ideas now need to be explored.

LIST OF ABBREVIATIONS

ABO	air-breathing organ
f_H	heart rate
f_R	ventilation frequency
f_{RA}	aerial respiration frequency
Pa	arterial pressure
Pbuccal	buccal pressure
P_{CO_2}	partial pressure of CO_2
P_{WO_2}	partial pressure of O_2 in water
T_{RA}	time spent in aerial respiration
V_{AMP}	ventilation amplitude
V_{TOT}	total ventilation

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the São Paulo State Research Foundation – FAPESP (Proc. 98/13534-1 and 00/12382-5), The National Council for the Development of Science and Technology – CNPq (Proc. 462594/00-9 and 304903/03-5) and the NSERC of Canada.

REFERENCES

Aota, S., Holmgren, K. D., Gallaugher, P. and Randall, D. J. (1990). A possible role for catecholamines in the ventilatory responses associated with internal acidosis or external hypoxia in rainbow trout. *J. Exp. Biol.* **151**, 57-70.

Axelsson, M. and Fritsche, R. (1994). Cannulation techniques. In *Biochemistry and Molecular Biology of Fishes. Analytical Techniques* (ed. P. W. Hochachka and T. P. Mommsen), pp. 17-36. Amsterdam: Elsevier.
Babiker, M. M. (1979). Respiratory behaviour, oxygen consumption and relative dependence on aerial respiration in the African lungfish (*Protopterus anectens*, Owen) and an air breathing teleost (*Clarius lazera*, C.). *Hydrobiologia* **65**, 177-187.
Bernier, N. J. and Randall, D. J. (1998). Carbon dioxide anaesthesi in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment. *J. Fish Biol.* **52**, 621-637.
Burleson, M. L. and Smatresk, N. J. (2000). Branchial chemoreceptors mediate ventilatory responses to hypercapnic acidosis in channel catfish. *Comp. Biochem. Physiol. A Physiol.* **125**, 403-414.
Butler, P. J. and Taylor, E. W. (1971). Responses of the dogfish (*Scyliorhinus canicula* L.) to slowly induced and rapidly induced hypoxia. *Comp. Biochem. Physiol. A Physiol.* **39**, 307-323.
Butler, P. J., Taylor, E. W. and Short, S. (1977). The effect of sectioning cranial nerves V, VII, IX and X on the cardiac response of the dogfish, *Scyliorhinus canicula* to environmental hypoxia. *J. Exp. Biol.* **69**, 233-245.
Carter, G. S. and Beadle, L. C. (1931). The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in fishes. *J. Linn. Soc. Lond. Zool.* **37**, 327-368.
Crocker, C. E., Farrell, A. P., Gamperl, A. K. and Cech, J. J., Jr (2000). Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Am. J. Physiol.* **279**, R617-R628.
Delaney, R. G., Lahiri, S. and Fishman, A. P. (1974). Aestivation of the African lungfish *Protopterus aethiopicus*: cardiovascular and respiratory functions. *J. Exp. Biol.* **61**, 111-128.
Delaney, R. G., Shub, C. and Fishman, A. P. (1976). Haematologic observations on the aquatic and aestivating African lungfish *Protopterus aethiopicus. Copeia* **1976**, 423-434.
Delaney, R. G., Lahiri, S., Hamilton, R. and Fishman, A. P. (1977). Acid–base balance and plasma composition in the aestivating lungfish (*Protopterus*). *Am. J. Physiol.* **232,** R10-R17.
Farrell, A. P. (1978). Cardiovascular events associated with air breathing in two teleosts, *Hoplerythrinus unitaeniatus* and *Arapaima gigas*. *Can. J. Zool.* **56**, 953-958.
Florindo, L. H., Reid, S. G., Kalinin, A. L., Milsom, W. K. and Rantin, F. T. (2004). Cardiorespiratory reflexes and aquatic surface respiration in the neotropical fish tambaqui (*Colossoma macropomum*): acute responses to hypercarbia. *J. Comp Physiol. B Biochem. Syst. Environ. Physiol.* **174**, 319-328.
Gilmour, K. M. (2001). The CO_2/pH ventilatory drive in fish. *Comp. Biochem. Physiol. A Physiol.* **130**, 219-240.
Gilmour, K. M. and Perry, S. F. (1994). The effects of hypoxia, hyperoxia or hypercapnia on the acid–base disequilibrium in the arterial blood of rainbow trout. *J. Exp. Biol.* **192**, 269-284.
Gilmour, K. M. and Perry, S. F. (1996). Effects of metabolic acid–base disturbances and elevated catecholamines on the acid-base disequilbrium in the arterial blood of rainbow trout. *J. Exp. Biol.* **274**, 281-290.
Gilmour, K. M. and Perry, S. F. (2007). Branchial chemoreceptor regulation of cardiorespiratory function. In *Fish Physiology*, Vol. 25, *Sensory Systems Neuroscience* (ed. B. Zielinski and T. J. Hara), pp. 97-151. San Diego: Academic Press.
Gilmour, K. M., Milsom, W. K., Rantin, F. T., Reid, S. G. and Perry, S. F. (2005). Cardiorespiratory responses to hypercarbia in tambaqui (*Colossoma macropomum*): chemoreceptor orientation and specificity. *J. Exp. Biol.* **208**, 1095-1107.
Glass, M. L. and Rantin, F. T. (2009). Gas exchange and control of respiration in air-breathing teleost fish. In *Cardio-Respiratory Control in Vertebrates – Comparative and Evolutionary Aspects* (ed. M. L. Glass and S. C. Wood), pp. 99-119. Berlin: Springer-Verlag.
Gonzalez, C., Almaraz, L., Obeso, A. and Rigual, R. (1994). Carotid body chemoreceptors: from natural stimuli to sensory discharges. *Physiol. Rev.* **74**, 829-898.
Graham, J. B. (1997). *Air-breathing Fishes: Evolution, Diversity and Adaptation*, 299 pp. San Diego: Academic Press.
Graham, J. B. and Baird, T. A. (1982). The transition to air breathing in fishes: I. Environmental effects on the facultative air breathing of *Ancistrus chagresi* and *Hypostomus plecostomus* (Loricariidae). *J. Exp. Biol.* **96**, 53-67.
Graham, M. S., Turner, J. D. and Wood, C. M. (1990). Control of ventilation in the hypercapnic skate, *Raja ocellata*. I. Blood and extradural fluid chemistry. *Respir. Physiol.* **80**, 259-277.
Hedrick, M. S., Burleson, M. L., Jones, D. R. and Milsom, W. K. (1991). An examination of central chemosensitivity in an air-breathing fish (*Amia calva*). *J. Exp. Biol.* **155**, 165-174.
Heisler, N., Toews, D. P. and Holeton, G. F. (1988). Regulation of ventilation and acid–base status in the elasmobranch *Scyliorhinus stellaris* during hyperoxia induced hypercapnia. *Respir. Physiol.* **71**, 227-246.
Hempleman, S. C., Powell, F. L. and Prisk, G. K. (1992). Avian arterial chemoreceptor responses to steps of CO_2 and O_2. *Respir. Physiol.* **90**, 325-340.
Hughes, G. M. and Shelton, G. (1962). Respiratory mechanisms and their nervous control in fish. *Adv. Comp. Physiol. Biochem.* **1**, 275-364.
Janssen, R. G. and Randall, D. J. (1975). The effects of changes in pH and PCO_2 in bood and water on breathing in rainbow trout, *Salmo gairdneri. Respir. Physiol.* **25**, 235-245.
Jesse, M. J., Shub, C. and Fishman, A. P. (1967). Lung and gill ventilation of the African lungfish. *Respir. Physiol.* **3**, 267-287.
Johansen, K. (1966). Air breathing in the teleost *Symbranchus marmoratus*. *Comp. Biochem. Physiol.* **18**, 383-395.
Johansen, K. and Lenfant, C. (1968). Respiration in the African lungfish, *Protopterus aethiopicus*. II. Control of breathing. *J. Exp. Biol.* **49**, 453-468.
Johansen, K., Lenfant, C. and Grigg, G. C. (1967). Respiratory control in the lungfish, *Neoceratodus fosteri* (Krefft). *Comp. Biochem. Physiol.* **20**, 835-854.
Johansen, K., Lenfant, C., Schmidt-Nielsen, K. and Petersen, J. A. (1968). Gas exchange and control of breathing in the electric eel, *Electrophorus electricus*. *Z. Vergl. Physiol.* **61**, 137-163.
Johansen, K., Hanson, D. and Lenfant, C. (1970). Respiration in a primitive air breather, *Amia calva*. *Respir. Physiol.* **9**, 162-174.
Kent, B. and Peirce, E. C. I. (1978). Cardiovascular responses to changes in blood gases in dogfish shark, *Squalus acanthias*. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* **60**, 37-44.
Kinkead, R. and Perry, S. F. (1991). The effects of catecholamines on ventilation in rainbow trout during external hypoxia or hypercapnia. *Respir. Physiol.* **84**, 77-92.
Kramer, D. L., Lindsey, C. C., Moodie, G. E. E. and Stevens, E. D. (1978). The fishes and aquatic environments of the Central Amazon Basin, with particular reference to respiratory patterns. *Can. J. Zool.* **56**, 717-729.
Kusakabe, T. (2002). Carotid labyrinth of amphibians. *Microsc. Res. Tech.* **59**, 207-226.
Lomholt, J. P. and Johansen, K. (1974). Control of breathing in *Amphipnous cuchia*, an amphibious fish. *Respir. Physiol.* **21**, 325-340.
Lopes, J. M., de Lima Boijink, C., Florindo, L.-H., Leite, C. A. C., Kalinin, A. L., Milsom, W. K. and Rantin, F. T. (2010). Hypoxic cardiorespiratory reflexes in the facultative air-breathing jeju (*Hoplerythrinus unitaeniatus*): role of branchial O_2 chemoreceptors. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* Epub ahead of print.
McKendry, J. E. and Perry, S. F. (2001). Cardiovascular effects of hypercapnia in rainbow trout (*Oncorhynchus mykiss*): a role for externally oriented chemoreceptors. *J. Exp. Biol.* **204**, 115-125.
McKendry, J. E., Milsom, W. K. and Perry, S. F. (2001). Branchial CO_2 receptors and cardiorespiratory adjustments during hypercarbia in Pacific spiny dogfish (*Squalus acanthias*). *J. Exp. Biol.* **204**, 1519-1527.
McMahon, B. R. and Burggren, W. W. (1987). Respiratory physiology of intestinal air breathing in the teleost fish *Misgurnus anguillicaudatus*. *J. Exp. Biol.* **133**, 371-393.
Milsom, W. K. (1995a). Regulation of respiration in lower vertebrates: role of CO_2/pH chemoreceptors. In *Advances in Comparative and Environmental Physiology*, Vol. 22 (ed. N. Heisler), pp. 62-104. Berlin: Springer-Verlag.
Milsom, W. K. (1995b). The role of CO_2/pH chemoreceptors in ventilatory control. *Braz. J. Med. Biol. Res.* **28**, 1147-1160.
Milsom, W. K. (2002). Phylogeny of CO_2/H^+ chemoreception in vertebrates. *Resp. Physiol. Neurobiol.* **131**, 29-41.
Milsom, W. K., Reid, S. G., Rantin, F. T. and Sundin, L. (2002). Extrabranchial chemoreceptors involved in respiratory reflexes in the neotropical fish *Colossoma macropomum* (the tambaqui). *J. Exp. Biol.* **205**, 1765-1774.
Nattie, E. (1999). CO_2, brainstem chemoreceptors and breathing. *Prog. Neurobiol.* **59**, 299-331.
Neville, C. M. (1979). Ventilatory responses of rainbow trout (*Salmo gairdneri*) to increased H^+ ion concentration in blood and water. *Comp. Biochem. Physiol. A Physiol.* **63**, 373-376.
Oliviera, R. D., Lopes, J. M., Sanches, J. R., Kalinin, A. L., Glass, M. L. and Rantin, F. T. (2004). Cardiorespiratory responses of the facultative air-breathing fish, jeju, *Hoplerythrinus unitaeniatus* (Teleostei, Erythrinidae), exposed to graded ambient hypoxia. *Comp. Biochem. Physiol. A Physiol.* **139**, 479-485.
Peers, C. and Buckler, K. J. (1995). Transduction of chemostimuli by the type I carotid body cell. *J. Membr. Biol.* **144**, 1-9.
Perry, S. F. and Gilmour, K. M. (1996). Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). *J. Exp. Biol.* **199**, 2105-2118.
Perry, S. F. and Gilmour, K. M. (2002). Sensing and transfer of respiratory gases at the fish gill. *J. Exp. Zool.* **293**, 249-263.
Perry, S. F. and McKendry, J. E. (2001). The relative roles of external and internal CO_2 *versus* H^+ in eliciting the cardiorespiratory responses of *Salmo salar* and *Squalus acanthias* to hypercarbia. *J. Exp. Biol.* **204**, 3963-3971.
Perry, S. F. and Reid, S. G. (2002). Cardiorespiratory adjustments during hipercarbia in rainbow trout *Oncorhynchus mykiss* are initiated by external CO_2 receptors on the first gill arch. *J. Exp. Biol.* **205**, 3357-3365.
Perry, S. F. and Wood, C. M. (1989). Control and coordination of gas transfer in fishes. *Can. J. Zool.* **67**, 2961-2970.
Perry, S. F., Fritsche, R., Hoagland, T., Duff, D. W. and Olson, K. R. (1999). The control of blood pressure during external hypercapnia in the rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **202**, 2177-2190.
Perry, S. F., Gilmour, K. M., Swenson, E. R., Vulesevic, B., Chew, S. F. and Ip, Y. K. (2005). An investigation of the role of carbonic anhydrase in aquatic and aerial gas transfer in the African lungfish (*Protopterus dolloi*). *J. Exp. Biol.* **208**, 3805-3815.
Perry, S. F., Euverman, R., Wang, T. A. M., Chew, S. F., Ip, Y. K. and Gilmour, K. M. (2008). Control of breathing in the African lungfish (*Protopterus dolloi*); a comparison of aquatic and cacooned (terrestrialized) animals. *Respir. Physiol. Neurobiol.* **160**, 8-17.
Rantin, F. T. and Kalinin, A. L. (1996). Cardiorespiratory function and aquatic surface respiration in *Colossoma macropomum* exposed to graded and acute hypoxia. In *Physiology and Biochemistry of the Fishes of the Amazon* (ed. A. L. Val, V. M. F. A. Val and D. J. Randall), pp. 169-180. Manaus, Brazil: Editora do INPA.
Rantin, F. T., Guerra, C. R., Kalinin, A. L. and Glass, M. L. (1998). The influence of aquatic surface respiration (ASR) on cardio-respiratory function of the serrasalmid fish *Piaractus mesopotamicus*. *Comp. Biochem. Physiol.*. **119A**, 991-997.
Reid, S. G., Sundin, L., Kalinin, A. L., Rantin, F. T. and Milsom, W. K. (2000). Cardiovascular and respiratory reflexes in the tropical fish, traíra (*Hoplias malabaricus*): CO_2/pH chemoresponses. *Respir. Physiol.* **120**, 47-59.
Sanchez, A. P. and Glass, M. L. (2001). Effects of environmental hypercapnia on pulmonary ventilation of the South American lungfish. *J. Fish Biol.* **58**, 1181-1189.
Sanchez, A. P., Hoffmann, A., Rantin, F. T. and Glass, M. L. (2001). Relationship between cerebro-spinal fluid pH and pulmonary ventilation of the South American lungfish, *Lepidosiren paradoxa* (Fitz.). *J. Exp. Zool.* **290**, 421-425.

Sanchez, A. P., Giusti, H., Bassi, M. and Glass, M. L. (2005). Acid–base regulation in the South American lungfish *Lepidosiren paradoxa*: Effects of prolonged hypercarbia on blood gases and pulmonary ventilation. *Physiol. Biochem. Zool.* **78**, 908-915.

Sioli, H. (1984). The Amazon and its main affluents: hydrography, morphology of the river courses, and river types. In *The Amazon: Liminology and Landscape Ecology of a Mighty Tropical River and its Basin* (ed. H. Sioli), pp. 127-165. Boston: Dr W. Junk Publisher.

Smatresk, N. J. (1990). Chemoreceptor modulation of endogenous respiratory rhythms in vertebrates. *Am. J. Physiol.* **259**, R887-R897.

Smith, H. W. (1930). Metabolism of the lungfish *Protopterus aethiopicus*. *J. Biol. Chem.* **88**, 97-130.

Smith, D. G. and Gannon, B. J. (1978). Selective control of branchial arch perfusion in an air-breathing Amazonian fish *Hoplerythrinus unitaeniatus*. *Can. J. Zool.* **56**, 959-964.

Smith, F. M. and Jones, D. R. (1982). The effect of changes in blood oxygen-carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* **97**, 325-334.

Soncini, R. and Glass, M. L. (2000). Oxygen and acid–base status related drives to gill ventilation in carp. *J. Fish Biol.,* **56**, 528-541.

Spix, J. B. and Agassiz, L. (1829). Selecta genera et species piscium quos in itinere per Brasiliam annos MDCCCXVII-MDCCCXX jussu et auspiciis Maximiliani Josephi I colleget et pingendso curavit. Monachii. Part I. Selecta Piscium Brasiliam, 82 pp.

Stevens, E. D. and Holeton, G. F. (1978). The partitioning of oxygen uptake from air and from water by erythrinids. *Can. J. Zool.* **56**, 965-969.

Sundin, L., Reid, S. G., Kalinin, A. L., Rantin, F. T. and Milsom, W. K. (1999). Cardiovascular and respiratory reflexes: the tropical fish, traíra (*Hoplias malabaricus*) O_2 chemoresponses. *Respir. Physiol.* **116**, 181-199.

Sundin, L., Reid, S. G., Rantin, F. T. and Milsom, W. K. (2000). Branquial receptors and cardiorespiratory reflexes in the neotropical fish, Tambaqui (*Colossoma macropomum*). *J. Exp. Biol.* **203**, 1225-1239.

Thomas, S. (1983). Changes in blood acid–base balance in trout (*Salmo gairdneri* Richardson) following exposure to combined hypoxia and hypercapnia. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **152**, 53-57.

Thomas, S. and Le Ruz, H. (1982). A continuous study of rapid changes in blood acid–base status of trout during variations of water PCO_2. *J. Comp. Physiol.* **148**, 123-130.

Todd, E. S. (1972). Hemoglobin concentration in a new air-breathing fish. *Comp Biochem. Physiol. A Physiol.* **42**, 569-573.

Vulesevic, B., McNeill, B. and Perry, S. F. (2006). Chemoreceptor plasticity and respiratory acclimation in the zebrafish *Danio rerio*. *J. Exp. Biol.* **209**, 1261-1273.

West, N. H. and Van Vliet, B. N. (1993). Sensory mechanisms regulating the cardiovascular and respiratory systems. In *Environmental Physiology of the Amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 151-182. Chicago: The University of Chicago Press.

Wilson, R. J. A., Harris, M. B., Remmers, J. E. and Perry, S. F. (2000). Evolution of air-breathing and central CO_2/H^+ respiratory chemosensitivity: new insights from and old fish? *J. Exp. Biol.* **203**, 3505-3512.

Wood, C. M. and Munger, R. S. (1994). Carbonic anhydrase injection provides evidence for the role of blood acid-base status in stimulating ventilation after exhaustive exercise in rainbow trout. *J. Exp. Biol.* **194**, 225-253.

Wood, C. M. and Perry, S. F. (1985). Respiratory, circulatory and metabolic adjustments to exercise in fish. In *Circulation, Respiration and Metabolism* (ed. R. Gilles), pp. 2-22. Berlin: Springer-Verlag.

Wood, C. M., Turner, J. D., Munger, R. S. and Graham, M. S. (1990). Control of ventilation in the hypercapnic skate *Raja ocellata*: II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Respir. Physiol.* **80**, 279-298.